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Thiago A.S. Gilio
USDA-ARS

Oscar P. Hurtado-Gonzales
USDA-ARS

G. Valentini
Universidade Estadual de Maringá

Sandra A. L. Castro
Universidade Estadual de Maringá

Haroldo T. Elias
EPAGRI

See next page for additional authors

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Authors

Thiago A.S. Gilio, Oscar P. Hurtado-Gonzales, G. Valentini, Sandra A. L. Castro, Haroldo T. Elias, Qijian Song, Maria Gonçalves-Vidigal, and M. A. Pastor-Corrales

FINE MAPPING THE BROAD SPECTRUM ANTHRACNOSE RESISTANCE GENE IN AMENDOIM CAVALO

Gilio, T.A.S.¹, Oscar P. Hurtado-Gonzales², Giseli Valentini¹, Sandra A.L. Castro¹, Haroldo T. Elias³, Qijian Song², Maria Gonçalves-Vidigal¹, and M.A. Pastor-Corrales²

¹Universidade Estadual de Maringá, PR, Brazil, Departamento de Agronomia; ²Soybean Genomics and Improvement Laboratory, ARS-USDA, Beltsville, MD 20705; ³EPAGRI

INTRODUCTION: The Andean common bean landrace Amendoim Cavalo (AC) is resistant to races 2, 7, 9, 19, 23, 39, 55, 65, 73, 89, 1545, 2047 and 3481 of *Colletotrichum lindemuthianum* (Nanami et al, 2014). None of the common bean anthracnose resistance genes known to date, were resistant to all 13 races mentioned above, to which AC was resistant. The resistance in AC is conferred by a single and dominant gene (*Co-AC*) that is independent of the other known genes (Nanami et al., 2014 and Gilio et al, 2016). The AC locus has been located in the lower arm of chromosome Pv01 of common bean (Gilio et al., 2016). The objective of this study was to use fine mapping to locate the position *Co-AC* locus in the common bean genome.

MATERIALS AND METHODS: To map the anthracnose resistance gene in AC, 110 F₂ seedlings from the cross PI 207262 (S) x AC (R) were inoculated with the race 3481 of *C. lindemuthianum*. Bulk segregant analysis (BSA) was performed using one susceptible and one resistant bulk, and the parents. Each bulk consisted of equal amounts of DNA from eight plants. To avoid including heterozygous resistance plants in the resistant bulk, F_{2:3} families were phenotypically evaluated using race 3481 of *C. lindemuthianum* to identify heterozygous resistant plants. DNA samples of the two bulks and the parents were screened with the BARCBEAN6K_3 BeadChip containing 5,398 SNPs following the Infinium HD Assay Ultra Protocol. SNP alleles were called using the GenomeStudio Genotyping Module v1.8.4 software. All allele calls were visually inspected. Positive hits for BSA were recorded when a SNP was polymorphic between PI 207262 and AC, and the susceptible bulk clustered with PI 207262 and the resistant bulk clustered with AC. The candidate region containing the *Co-AC* resistant locus was targeted with SSR and KASP markers for genetic mapping in the F₂ population. Genetic distances were estimated using the software JoinMap 4.0. To fine mapping *Co-AC* locus, F_{2:3} families were selected based on the recombination detected between the flanking KASP markers ss56 and ss92 at F₂ level. The number of plants per F_{2:3} families varied from 1 to 15 plants. F_{2:3} families showing recombination between markers ss56 and ss92 were selected for additional phenotyping with race 3481 of *C. lindemuthianum* and genotyping with newly designed KASP markers to narrow the region of the *Co-AC* locus. Candidate genes were verified in the reference genome G19833 with Phytozome 11.0 (www.phytozome.org).

RESULTS AND DISCUSSION: A total of 21 SNPs resulted positive in the BSA with the SNP BARCBEAN6K_3 BeadChip. The physical location of the positive associated SNPs was on linkage group Pv 01 between 48448199 bp and 50301592 bp, spanning a region of 1.85 Mbps. The results of the mapping analysis of F₂ population from cross Amendoim Cavalo x PI 207262 with six SSRs and four KASP markers showed that the resistance *locus* in AC was located between the markers BARCPVSSR01342 (1.1 cM) and ss92 (1.3 cM). The closest KASP markers in this genetic map was ss55 and ss56 (3.4 cM) upstream and ss92 (1.3 cM) downstream (Figure 1A). The flanking KASP markers ss56 and ss92, were chosen to screen 62 F_{2:3} families

(with genotype heterozygous or recombinants) to explore additional recombinants events. KASP marker ss56 (49,895,862 bp) and the KASP marker ss92 (50,527,176 bp), flanking a physical region of 631 kbp (Figure 1B). A total of 700 F_{2:3} plants were inoculated with race 3481 of *C. lindemuthianum* and genotyped with the flanking KASP markers. From 700 F_{2:3} plants, 86 had genotypic recombinants events, between the flanking markers (ss56 and ss92). These 86 F_{2:3} plants were selected for fine mapping and the region between the flanking markers (631,314 bp) were saturated with seven KASP markers. Based on the recombination events we determined that the AC resistance locus was positioned between KASP markers ss102 (50,377,247 bp) and ss95 (50,442,472 bp) (Figure 1B), spanning a small region of 65.22 Kbp. Nine candidate genes were found in the reference genome (Figure 1C). These results demonstrated that the *Co-AC* anthracnose resistance gene was different from *Co-x* present in Pv01 (physical region 50,332,737 to 50,322,583 bp) (Richard et al., 2014).

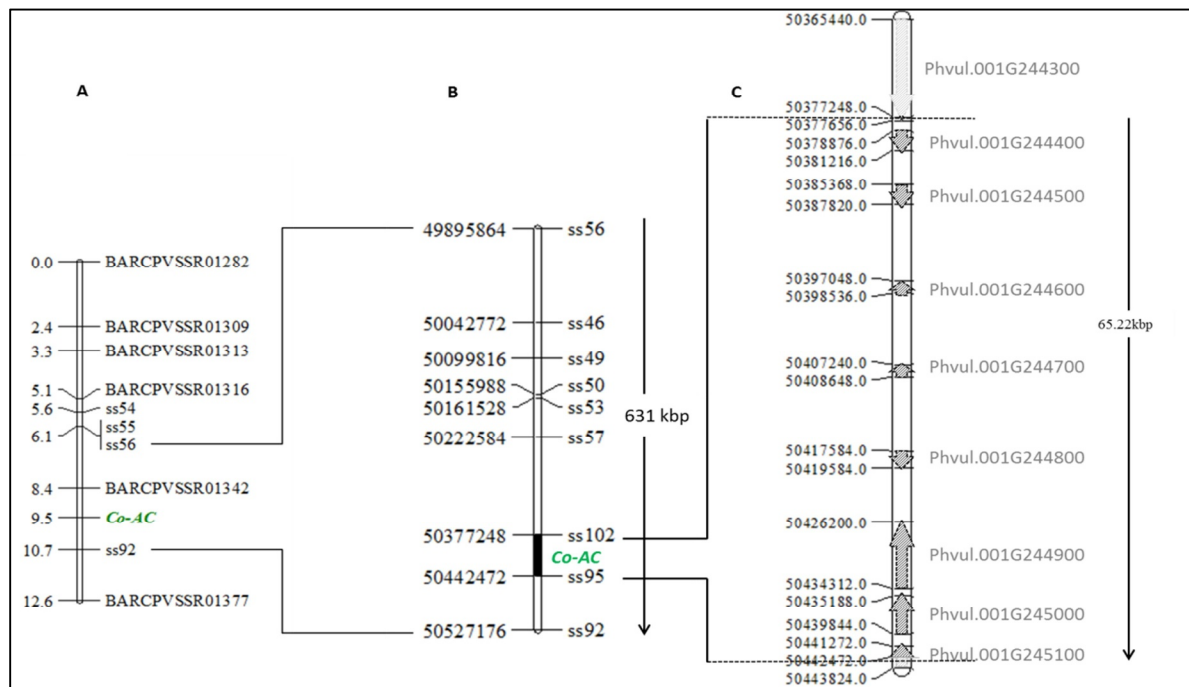


Figure 1. Genetic map of common bean linkage group Pv01 containing the *Co-AC* locus, SSRs and, SNP KASP markers: 1A, flanking KASP markers ss92 and ss56 were used to find recombinants genotypes plants in F_{2:3}; 1B, seven KASP were used to fine mapping the *Co-AC* locus to 65.22 Kbp; 1C, nine candidate genes were found between the flanking markers ss102 and ss95 according to the reference genome G 19833.

REFERENCES

- Gilio, T.A.S., D.S.Y. Nanami, M.C. Gonçalves-Vidigal, G. Valentini G., Q. Song Q., and M.A. Pastor-Corrales. 2016. Further characterization of the broad-spectrum anthracnose resistance in Andean common bean amendoim cavalo. *Annu. Rep. Bean Improv. Coop.* 59:127-128.
- Nanami, D.S.Y., A.A.T. Frias, S.A.L. Castro, J.C.F. Elias, G.F. Lacanallo, and M.C. Gonçalves-Vidigal. 2014. Inheritance and allelic relationships of anthracnose resistance in common bean Amendoim Cavalo. *Annu. Rep. Bean Improv. Coop.* 57:165-166.
- Richard, M.M., S. Pflieger, M. Seignac, V. Thareau, S. Blanchet, Y. Li, S. Jackson, and V. Geffroy. 2014. Fine mapping of *Co-x*, an anthracnose resistance gene to a highly virulent strain of *Colletotrichum lindemuthianum* in common bean. *Theor Appl Genet* 127:1653–1666.